SPECIFICATION AMENDMENT

Please replace the paragraphs found at page 108, line 11, to page 113, line 10, with the following:

EXAMPLE 1

Isolation of a *Zea mays* Cytoplasmic, Pedicel-Specific Glutamine Synthetase GS_{1-2} Promoter

Inverse PCR was used as described herein to isolate the maize GS_{1-2} promoter from *Zea mays* 01IBH2 (a DeKalb proprietary inbred line) genomic DNA. Approximately 1 μg of maize genomic DNA was digested with PstI restriction enzyme according to manufacturer's recommendations (Roche Molecular Biochemicals, Indianapolis IN). Following an overnight digestion at 37^0C, the digestion reaction was purified using an Amicon ~~Micropure~~MICROPURE-EZ kit and the purified, digested DNA was then concentrated to a final volume of approximately 8 μl using an Amicon ~~Microcon~~MICROCON apparatus, both used as per manufacturer's recommendations (Millipore Corporation, Bedford MA).

Approximately 125 ng (1 μl) of the digested genomic maize DNA was then self-ligated in the presence of T_4 DNA ligase, following the procedure of a Roche Rapid DNA ligation kit (Roche Molecular Biochemicals, Indianapolis IN). The ligation was carried out overnight, approximately 18 hours, at 16^0C. The completed ligation reaction was heat treated at 70^0C for 15 minutes, diluted by the addition of 70 μl of distilled, deionized water and stored at 4^0C.

Oligonucleotide primers for a primary inverse PCR reaction were designed based upon the maize glutamine synthetase GS_{1-2} sequence disclosed by Li *et al.*, (1993; accession number X65927). One skilled in the art would realize that other primers could be designed using this sequence to obtain similar results. Primers as239 (SEQ ID NO:1; Figure 1) and s350 (SEQ ID NO:2; Figure 1) comprised the following sequences:

as239 $^{5'}$ TCA TCA ACA GGT CCG GAC AG $^{3'}$

s350 $^{5'}$ GGA AGG GGC AGA ACA TAC TG $^{3'}$

One μl of the diluted ligation reaction was used as the DNA template in the primary PCR reaction. The reaction comprised primers as239 (SEQ ID NO:1) and s350 (SEQ ID NO:2) in a

mixture containing Buffer 3, nucleotides and thermostable DNA polymerase according to the conditions outlined by an ~~Expand Long~~ EXPAND LONG PCR kit (Roche Molecular Biochemicals, Indianapolis IN). A 7-step PCR™ program was utilized:

1. 94^{0}C for 25 seconds
2. 72^{0}C for 3 minutes
3. go to step 1 for 7 cycles
4. 94^{0}C for 25 seconds
5. 67^{0}C for 3 minutes
6. go to step 4 for 32 cycles
7. 67^{0}C for 7 minutes

Primers for the secondary inverse PCR reaction were designed based upon the maize glutamine synthetase GS_{1-2} sequence disclosed by Li *et al.,* (1993; accession number X65927). The second set of primers were "nested" primers, that is, they hybridize to the glutamine synthetase GS_{1-2} sequence at locations contained wholly within the PCR product produced in the primary PCR reaction. One skilled in the art would realize that other nested primers, such as primers that overlap with the primary PCR primers, could be designed using this sequence to obtain similar results. Primers as122 (SEQ ID NO:3; Figure 1) and s400 (SEQ ID NO:4; Figure 1) comprised the following sequences:

as122 $^{5'}$ AGG TCG GAG AGC AGA GCC AT $^{3'}$

s400 $^{5'}$ GAG CCG ATC CCG AGC AAC AA $^{3'}$

One µl of the primary PCR reaction was diluted into 70 ul of distilled, deionized water and used as template DNA in the secondary PCR reaction. The reaction comprised primers as122 (SEQ ID NO:3) and s400 (SEQ ID NO:4) in a mixture containing Buffer 3, nucleotides and thermostable DNA polymerase according to the conditions outlined by the ~~Expand Long~~ EXPAND LONG PCR kit (Roche Molecular Biochemicals, Indianapolis IN). A 5-step PCR program was utilized:

1. 95^{0}C for 1 minute
2. 95^{0}C for 20 ~~seoends~~seconds
3. 68^{0}C for 3 minutes
4. go to step 2 for 30 cycles

5. 68^0C for 7 minutes

An aliquot of the secondary PCR reaction was separated on an agarose gel, visualized with ethidium bromide stain and was shown to have produced a single band of PCR product of approximately 2.7 kilobases (Kb) in size.

An aliquot of the secondary PCR reaction was ligated into the pGEM-T-[[Easy]]EASY vector according to the manufacturer's recommendations (Promega, Madison WI). An aliquot of the ligation reaction was used to transform competent DH5α *E. coli* cells which were plated onto solid media with the appropriate supplements to allow for ampicillin resistance and blue/white colony selection. In this type of selection, bacterial cells transformed with plasmids containing an inserted fragment of DNA are typically white in color, while cells containing plasmids that do not have additional DNA are typically blue in color. Several white colonies were selected for further analysis. One of skill in the art would realize that alternate standard molecular biology methods may be employed to clone a desired PCR DNA fragment (Sambrook and Russell, 2001; Ausubel *et al.*, 2001).

Bacterial cells from the white colonies were mixed with 10-50 µl of water, preferably 20-40 µl of water and most preferably, 30 µl of water, heated to 100^0C for approximately 3 minutes and 1 µl of the slurry was used for PCR. The reaction comprised primer as122 (SEQ ID NO:3) and primer s400 (SEQ ID NO:4) in a mixture containing Buffer 3, nucleotides and thermostable DNA polymerase according to the conditions outlined by the ~~Expand Long~~ EXPAND LONG PCR kit (Roche Molecular Biochemicals, Indianapolis, IN). A 5-step PCR program was utilized:

1. 95^0C for 1 minute
2. 95^0C for 20 ~~seocnds~~seconds
3. 68^0C for 3 minutes
4. go to step 2 for 30 cycles
5. 68^0C for 7 minutes

An aliquot of the PCR reaction was separated on an agarose gel, visualized with ethidium bromide stain and was shown to have produced a single band of PCR product approximately 2.7 kilobases (Kb) in size.

To further confirm the identity of the cloned fragment as being associated with GS_{1-2}, it was desired to design a new oligonucleotide primer specific to the GS_{1-2} 5' untranslated region. Using BLAST analysis against public sequences from a variety of organisms including corn (Altschul *et al.*, 1997) it was determined that the terminal 66 base pairs at the 5' end of the untranslated region of GS_{1-2} as reported by Li *et al.*, (1993) were unique to the GS_{1-2} gene from maize and showed little to no significant homology to other sequences in the databases. Thus, a primer may be designed to hybridize to only to the GS_{1-2} 5' untranslated region, and that would not hybridize with sequences reported for the five other maize GS genes. Primer s1 was designed to this unique region and comprised the following sequence (SEQ ID NO:5; Figure 1):

s1 $^{5'}$ CGA AAG CAC ACA CGG ATC AA $^{3'}$

The PCR reaction comprised primers as122 (SEQ ID NO:3) and s1 (SEQ ID NO:5) in a mixture containing Buffer 3, nucleotides and thermostable DNA polymerase according to the conditions outlined by the ~~Expand Long~~ EXPAND LONG PCR kit (Roche Molecular Biochemicals, Indianapolis IN). A 5-step PCR program was utilized:

1. 95^{0}C for 1 minute
2. 95^{0}C for 20 ~~seoends~~seconds
3. 68^{0}C for 3 minutes
4. go to step 2 for 30 cycles
5. 68^{0}C for 7 minutes

An aliquot of the PCR reaction was separated on an agarose gel, visualized with ethidium bromide stain and was shown to have produced a single band of PCR product approximately 2.6 kilobases (Kb) in size.

The cloned PCR fragment was sequenced using SP_6 and T_7 primers hybridizing to the pGEM vector (Promega, Madison WI) as well as a series of primers which were designed to hybridize to internal sequences (SEQ ID NOS:6-17). The cloned PCR fragment was determined to be 2670 base pairs in length, 121 based pairs of which were found to overlap with 121 base pairs in the 5' untranslated region of the GS_{1-2} gene reported by Li *et al.*, (1993; accession number X65927) and as shown by sequence alignment using ~~Sequencher~~SEQUENCHER Software (version 4.0.5, Gene Codes Corporation, Ann Arbor, MI). A BLAST analysis of a 2.55 Kb subfragment of the putative GS_{1-2} promoter (described in Example 2) showed that sequences

from about base pair (bp) 100 to about bp 157, and from about bp 268 to about bp 400, showed homology to GS1-2 cDNA sequence (accession number X65927; Li *et al.*, 1993). Further BLAST analysis using default parameters did not reveal homology with any other sequence in the GenBank database. These data suggest that the cloned fragment of DNA produced by inverse PCR utilizing primers designed against a maize $GS_{1\text{-}2}$ sequence comprises the sequence for the maize $GS_{1\text{-}2}$ promoter.

EXAMPLE 2

Construction of $GS_{1\text{-}2}$ Promoter Containing Transformation Vectors

This example describes the construction of vector pMON65159. The pGEM vector comprising the putative $GS_{1\text{-}2}$ promoter fragment was digested with NcoI and PvuI (Roche Molecular Biochemicals, Indianapolis IN) which allowed removal of approximately 2.55 Kb of the cloned 2.7 Kb insert. The ends of the digested DNA were made blunt using the Stratagene Klenow Fill-In Kit (Stratagene, La Jolla CA). The products of the digestion were separated on an agarose gel and a slice containing the 2.55 Kb fragment with the putative $GS_{1\text{-}2}$ promoter was removed from the gel. The DNA was purified from the agarose gel using a ~~GenElute~~GENELUTE Agarose Spin column (Sigma Chemical Co., St. Louis, MO) as per manufacturer's recommendations.

Vector pGUS33, comprising the *uidA* screenable marker gene (Jefferson *et al.*, 1986), intron 1 from the rice actin 1 gene (McElroy *et al.*, 1990) and *pinII* 3' UTR (Graham *et al.*, 1986), as well as the 35S promoter, (Odell *et al.*, 1985), the NPT II selectable marker gene (Potrykus *et al.*, 1985) and *nos* 3' UTR (Bevan *et al.*, 1983), was digested with XhoI and BsteII (Roche Molecular Biochemicals, Indianapolis IN). The ends digested DNA were made blunt using the Stratagene Klenow Fill-In Kit (Stratagene, La Jolla CA). The filled-in ends of the pGUS33 backbone fragment, as well as other fragments from the digestion, were also dephosphorylated with calf alkaline intestinal phosphatase (Roche Molecular Biochemicals, Indianapolis IN). The approximately 7.8 Kb backbone fragment containing the *uidA* gene, intron and *pinII* 3'UTR and the 35S promoter, NPT II gene and *nos* 3' UTR, was isolated from an agarose gel slice using a ~~GenElute~~GENELUTE Agarose Spin column (Sigma Chemical Co., St. Louis, MO) as per manufacturer's recommendations. The $GS_{1\text{-}2}$ promoter DNA fragment and

the pGUS33 backbone fragment were ligated together using a Roche Rapid DNA ligation kit (Roche Molecular Biochemicals, Indianapolis IN) and ligation products transformed into competent *E. coli* cells.

Please replace the paragraph beginning at page 114, line 15, with the following paragraph:

DNA was introduced into maize cells using the electric discharge particle acceleration gene delivery device (U.S. Patent No. 5,015,580). The gold particle/DNA suspension was coated on ~~Mylar~~MYLAR sheets (Du Pont ~~Mylar~~MYLAR polyester film type SMMC2, aluminum coated on one side, over coated with PVDC co-polymer on both sides, cut to 18 mm square) by dispersion of 310 to 320 µl of the gold particle/DNA suspension on a sheet. After the gold particle suspension settled for one to three minutes, excess ethanol was removed and the sheets were air dried. Microprojectile bombardment of maize tissue was conducted as described in U.S. Patent No. 5,015,580. AC voltage may be varied in the electric discharge particle delivery device. For microprojectile bombardment of Hi-II or H99 pre-cultured immature embryos, 30% to 40% of maximum voltage was preferably used. Following microprojectile bombardment, tissue was cultured in the dark at 27°C.